METHOD AND DETECTOR FOR IDENTIFYING SUBTYPES OF HUMAN PAPILLOMA VIRUSES

FIELD OF THE INVENTION

The present invention relates to a method and a detector for detecting human papilloma viruses, and more particularly to a method and a detector for simultaneously detecting and identifying subtype of human papilloma viruses.

BACKGROUND OF THE INVENTION

Cervical cancer is the most common cancer in women. The consorts are often men with penile warts. Sexual activity appears to be an important predisposing factor of the epidemic disease and precancerous lesions. In early 5 to 10 years during the development of cervical cancer, cervical cells form cervical intraepithelial neoplasm.

Recently, in order to decrease the incidence of cervical cancer, Pap smear is used for the cervical cancer screening. However, the Pap smear has a false negative rate of about 30%~40%. In addition, it is known that more that 95% of cervical carcinoma tissue contain detectable DNA sequences for known varieties of the human papilloma virus (HPV). Hence, the combination of Pap smear and HPV detection for the cervical cancer screening is considered.

The applicant cooperates with the hospital to did the epidemiolory research in women cervical cancer by using Pap smear and HPV detection, wherein the HPV detection is proceeded by using polymerase chain reaction and nucleotide sequencing. There are 2424 women aged from 16 to 84 for the epidemiology research, wherein 1963 women provide the effective specimen. The research results are shown as follows.

1) 1.9% (37/1963) of the women have abnormal cytological smears.

2) 12.7% (244/1926) of the women with normal cytological smears but have HPV infection.

3) The HPV prevalence in the women with abnormal cytological smears is 51.4% (19/37) and positively relative to the degree of the abnormal cytological smears, wherein the incidence of abnormal non-typical squamous cells is 23.1%, the incidence of low abnormal epithelial cells is 41.7%, and the incidence of high abnormal epithelial cells is 75%.

4) The subtypes of human papilloma viruses detected in the specimens are HPV 52, HPV 58, HPV 70, HPV 16, HPV 18, HPV 68, HPV 33, HPV 66, HPV 35, HPV 37, HPV 54, HPV 59, HPV 67, HPV 72, HPV 69, HPV 82, HPV 39, HPV 31, HPV 32, HPV HLT7474-S, HPV 6, HPV CP8061, HPV 62, HPV CP8304, HPV 44, HPV 11, HPV 61, HPV 74, HPV 42 and HPV 43.

The conventional HPV detecting kits are only used for detecting 18 subtypes of human papilloma viruses including high risk HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59 and HPV 68, and detecting low risk HPV 6, HPV 11, HPV 42, HPV 43 and HPV 44.

According to the comparison of the epidemiology research and the conventional HPV detecting kits, various subtypes of human papilloma viruses contained in a specimen would not be identified by the conventional HPV detecting kits. In addition, the conventional HPV detecting kits are only used for detecting the high risk HPV and the low risk HPV, but not for identifying the HPV subtypes. Furthermore, the conventional HPV detecting kits lack the system control for checking the house-keep genes contained in a specimen.

In order to overcome the foresaid drawbacks of the conventional HPV detecting kits, the present invention provides a method and a detector for simultaneously detecting and identifying subtypes of human papilloma viruses contained in a sample.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a detector for simultaneously detecting and identifying subtypes of human papilloma viruses (HPV) contained in a sample.

In accordance with the present invention, the detector includes a carrier having a first part and a second part for carrying the sample thereon, a first oligonucleotide carried on said first part of the carrier, and a second oligonucleotide carried on the second part of the carrier, wherein the first and second oligonucleotides respectively hybridized with deoxyribonucleic acids contained in a first subtype of human papilloma virus and a second subtype of human papilloma virus for simultaneously detecting and identifying subtypes of human papilloma viruses.

Preferably, the carrier is made of nylon.

Preferably, the carrier is a glass plate.

Preferably, the first and second subtypes of human papilloma viruses are respectively selected from 38 subtypes of human papilloma viruses, wherein the sequence of the first oligonucleotide is selected from one of the sequence group corresponding to the first subtype of human papilloma virus and complementary sequences thereof, and the sequence of the second oligonucleotide is selected from one of the sequence group corresponding to the second subtype of human papilloma virus and complementary sequences thereof.

Preferably, the detector could be an oligonucleotide chip.

In another aspect of the present invention to provide a method for simultaneously detecting and identifying subtypes of human papilloma viruses contained in a sample.

In accordance with the present invention, the method includes steps of providing a first oligonucleotide and a second oligonucleotide for respectively hybridizing with a first and a second subtypes of human papilloma viruses, hybridizing deoxyribonucleic acid (DNA) contained in the sample with the first and second oligonucleotides, and removing nonhybridized DNA, thereby the subtypes of human papilloma viruses contained in the sample are detected and identified.

Preferably, the DNA contained in the sample is the product of polymerase chain reaction (PCR).

Preferably, the DNA contained in the sample has signaling substances labeled thereon.

Preferably, the signaling substance is biotin.

In addition, the method further includes a step of performing a reaction of biotin and avidin-alkalinephosphatase.

On the other hand, the signaling substances could be fluorescent substances. Preferably, the fluorescent substance is Cyanine 5.

It is another aspect of the present invention to provide a method for detecting a subtype of human papilloma viruses contained in a sample.

In accordance with present invention, the method includes steps of providing an oligonucleotide complementary to a sequence specific to the subtype of human papilloma viruses, hybridizing said oligonucleotide with deoxyribonucleic acid (DNA) contained in the sample, removing non-hybridized DNA contained in the sample, and detecting hybridized DNA

to show whether the subtype of human papilloma viruses contained in the sample.

The present invention may best be understood through the following descriptions with reference to the accompanying drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic view showing the detector according to the first preferred embodiment of the present invention;

Fig. 2 is a schematic view showing the detector according to the second preferred embodiment of the present invention;

Fig. 3(a) is a schematic view showing the detector according to the third preferred embodiment of the present invention;

Fig. 3(b) is a schematic view illustrating the subtype of human papilloma viruses identified by each dot shown in Fig. 3(a);

Fig. 4(a) is the electrophoresis result showing the analyzed products of the first polymerase chain reaction;

Fig. 4(b) is the electrophoresis result showing the analyzed products of the second polymerase chain reaction;

Fig. 4(c) is detecting result on the detectors of detecting the HPV positive clones according to the third preferred embodiment of the present invention;

Fig. 5 is a view showing the detecting result on the detectors of detecting samples according to the third preferred embodiment of the present invention;

Fig. 6(a) is a schematic view showing the detector according to the fourth preferred embodiment of the present invention;

Fig. 6(b) is a schematic view illustrating the subtype of human papilloma viruses identified by each dot shown in Fig. 6(a);

Fig. 7(a) is a view showing the detector stained with SYBR Green II according to the fourth embodiment of the present invention; and

Fig. 7(b) is a view showing the detecting result on the detectors of detecting samples according to the fourth preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Please refer to Fig. 1. A detector 10 is the first embodiment of the present invention for simultaneously detecting and identifying the subtypes of human papilloma viruses contained in a sample. The detector 10 includes a carrier 11 and a dot array. The carrier 11 is a nylon membrane having the dot array 12 mounted thereon. Each dot in the dot array 12 is an oligonucleotide (15~30mer) for identifying a specific subtype of human papilloma viruses.

The sequences of the oligonucleotides provided by the present invention are specific to the epidemics of human papilloma viruses. The sequences of the oligonucleotides shown in Tables 1 to 38 are determined by the way of comparing DNA sequences of 97 subtypes of human papilloma viruses. Each table illustrates a plurality of oligonucleotides for identifying a specific subtype of human papilloma viruses.

Table 1. Sequences and loci of oligonucleotides for identifying HPV 11

Sequence No.	5'→ 3'	Locus in HPV 11
M1101	ATCTGTGTCTAAATC	6799 –6813
M1102	TCTGTGTCTAAATCTGCTAC	6800 – 6819
M1103	ATCTGTGTCTAAATCTGCTACATACA	6799 – 6824
M1104	TGCATCTGTGTCTAAATCTG	6796 – 6815
M1105	AAATCTGCTACATACACTAA	6809 – 6828
M1106	CTAAATCTGCTACATACACTA	6807 – 6827
M1107	CTACATACACTAATTCAGAT	6816 – 6835

M1108	TAGCATTACATTATCTGCAGAAG	6895 – 6917
M1109	TCCTTCTGTTTTGGAGGAC	6943 – 6961
M1110	TTTATCGCCTCCACCAAATGGTACAC	6973 – 6998
M1111	TTATAGATATGTACAGTCACAGGCC	7009 – 7033
M1112	ACCCACACCTGAAAAAGAAAAAC	7048 – 7070
M1113	GGCGGATGCTCATTATGCGACTG	1044 - 1066
M1114	ATATGTAAGTCCTATAAGCAATGTAG	1101 - 1126
M1115	CTAATGCAGTAGAAAGTGAGATAAGT	1127 - 1152
M1116	CACGGTTAGACGCCATTAAACTTACA	1154 - 1179
M1117	CACAGCCAAAAAAGGTAAAGCGACGG	1181 - 1206
M1118	GCAACGCAGGTAGAGAAACATGGCGA	1264 - 1289
M1119	GAAAATGGGGGAGATGGTCAGGAAAGGGAC	1294 - 1323
M1120	GACACAGGGAGGGACATAGAGGGTGA	1321 - 1346
M1121	ACATAGAGAGGCGGAAGCAGTAGACG	1356 - 1381
M1122	ACAGCACCCGAGAGCATGCAGACACA	1382 - 1407
M1123	TCAGGAATATTAGAATTACTAAAATG	1408 - 1433
M1124	GCTGTCATTTGTTGATTTA	1482 - 1500

Table 2. Sequences and loci of oligonucleotides for identifying HPV 16

Sequence No.	5'→ 3'	Locus in HPV 16
M1601	TATGTCATTATGTGCTGCCA	6659 – 6678
M1602	GTGCTGCCATATCTACTTCA	6670 – 6689
M1603	TGCCATATCTACTTC	6674 – 6688
M1604	TATCTACTTCAGAAACTACA	6679 – 6698
M1605	CTACTTCAGAAACTACATATAA	6682 – 6703
M1606	ATAAAAATACTAACTTTAAG	6700 – 6719
M1607	CAAAATAACCTTAACTGCAGACG	6773 – 6795
M1608	TTCCACTATTTTGGAGGAC	6821 – 6839
M1609	TCTACAACCTCCCCCAGGAGGCACAC	6851 – 6876
M1610	TTATAGGTTTGTAACCCAG	6887 – 6905
M1611	ACATACACCTCCAGCACCT	6923 – 6941
M1612	CCTTAAAAAATACACT	6956 – 6971
M1613	AGCAAAACAACATAGAGATGCAG	1089 - 1111
M1614	ACTTAGTGATATTAGTGGATGTGTAG	1145 - 1170
M1615	TAGTCCTAGATTAAAAGCTATATGTATAGA	1181 - 1210

M1616	GAAAAACAAAGTAGAGCTGCAAAAAG	1209 - 1234
M1617	CTCAGCAGATGTTACAGGTAGAAGGG	1285 - 1310
M1618	CCATGAGACTGAAACACCATGTAGTC	1313 - 1338
M1619	GGGGGTGGTTGCAGTCAGTACAGTAG	1356 - 1381
M1620	GTGGGGGAGAGGGTGTTAGTGAAAGA	1387 - 1412
M1621	CTATATGCCAAACACCACTTACAAAT	1417 - 1442
M1622	GTTATACGGGGTGAGTTTTTCAGAAT	1502 - 1527

Table 3. Sequences and loci of oligonucleotides for identifying HPV 18

Sequence No.	5'→ 3'	Locus in HPV 18
M1801	TTCTACACAGTCTCC	6650 – 6664
M1802	CAGTCTCCTGTACCTGGGCA	6657 – 6676
M1803	AGTCTCCTGTACCTGGGCAA	6658 – 6677
M1804	TCTCCTGTACCTGGGCAATATGA	6660 – 6682
M1805	CTGTACCTGGGCAATATGAT	6664 – 6683
M1806	ATGATGCTACCAAATTTAAG	6679 – 6698
M1807	TACTATTACTTTAACTGCAGATG	6752 – 6774
M1808	TAGCAGTATTTTAGAGGAT	6800 – 6818
M1809	TGTTCCCCCCCCCCCAACTACTAGTT	6830 – 6855
M1810	ATATCGTTTTGTACAATCTGTT	6866 – 6887
M1811	GGATGCTGCACCGGCTGAA	6905 – 6923
M1812	CTATGATAAGTTAAAG	6935 – 6950
M1813	GGTCCACAATGATGCACAAGTGT	1135 - 1157
M1814	CACAGAAAACAGTCCATTAGGGGAGC	1192 - 1217
M1815	GCTGGAGGTGGATACAGAGTTAAGTC	1219 - 1244
M1816	AGTGGGCAGAAAAAGGCAAAAAGGCGG CTG	1271 - 1300
M1817	CACAGATTCAGGTAACTACAAATGGC	1347 - 1372
M1818	CAATGTATGTAGTGGCGGCAGTACGG	1384 - 1409
M1819	GACAACGGGGGCACAGAGGGCAACAA	1418 - 1443
M1820	GTAGACGGTACAAGTGACAATAGCAA	1451 - 1476
M1821	CCACAATGTACCATAGCACAATTAAA	1493 - 1518
M1822	CACATATGGGCTATCATTTACAGATT	1573 - 1598

Table 4. Sequences and loci of oligonucleotides for identifying HPV 26

Sequence	5'→ 3'	Locus in

No.		HPV 26
M2601	TAGTACATTATCTGCAGCAT	6619 – 6638
M2602	ATTATCTGCAGCATC	6625 – 6639
M2603	TGCAGCATCTGCATCCACTC	6631 – 6650
M2604	GCATCTGCATCCACTCCATTTAAA	6635 – 6658
M2605	CTCCATTTAAACCATCTGAT	6648 – 6667
M2606	TAAAATAACACTTACAACAGATG	6727 – 6749
M2607	TGCCTCCATATTGGAGGAT	6775 – 6793
M2608	ACTAACCTTACCTCCCACTGCTAGTT	6805 – 6830
M2609	CTATAGGTTTATTAAAAACTCT	6841 – 6862
M2610	TAACGCCCCTCCTGTGCCA	6880 – 6898
M2611	AAAACAGGCAAATACAAAGGCAG	1093 - 1115
M2612	CTAGGTAGTCAGAACAGCCCGTTGCA	1139 - 1164
M2613	GCGACAGTCAGCAGAATACACACCAAG TAA	1194 - 1223
M2614	CAAAAGGAGAGCCGTGGACAGTGTAC	1237 - 1262
M2615	CCGTACAGGTAGATAAACAATATGAA	1305 - 1330
M2616	GCCTAGTGTGTGTAGTCAGGGGGGG	1345 - 1369
M2617	GCCTCAGTGGAAGATATCGATGTAGA	1376 - 1401
M2618	CAGTGTTACACAAATATGTGAATTAT	1414 - 1439
M2619	CAGTATATGGTGTAAGTTTTGCAGAA	1485 - 1510

Table 5. Sequences and loci of oligonucleotides for identifying HPV 31

Sequence No.	5'→ 3'	Locus in HPV 31
M3101	TGCAATTGCAAACAG	6592 – 6606
M3102	GCAATTGCAAACAGTGATAC	6593 – 6612
M3103	CAATTGCAAACAGTGATACT	6594 – 6613
M3104	GCAAACAGTGATACTACATTTAA	6599 – 6621
M3105	CTACATTTAAAAGTAGTAAT	6612 – 6631
M3106	CAAAATAACATTATCTGCAGACA	6691 – 6713
M3107	TCCTGCTATTTTGGAAGAT	6739 – 6757
M3108	ATTGACCACACCTCCCTCAGGTTCTT	6769 – 6794
M3109	CTATAGGTTTGTCACCTCACAG	6805 – 6826
M3110	AACTGCCCCCCAAAAGCCC	6844 – 6862
M3111	AGCGGAGGAACATGCAGAGGCTG	1083 - 1105
M3112	TTTAAGTGATATTAGTAGTTGTGTGG	1140 - 1165

M3113	AGTCCACGGTTAAAAGCTATATGCATAGAA	1177 - 1206
M3114	GACTCTTTGAACTTCCAGACAGCGGG	1232 - 1257
M3115	GCAGCAGATGGTACAGGTAGAGGAGC	1281 - 1306
M3116	AATGGTAGTGACGGGACACATAGTGA	1327 - 1352
M3117	CCAACACGTAATATATTGCAAGTGTT	1369 - 1394
M3118	GCAATGGTAAAGCTGCTATGTTAGGT	1403 - 1428
M3119	TTATATGGTGTAAGTTTTATGGAAC	1441 - 1465

Table 6. Sequences and loci of oligonucleotides for identifying HPV 32

Sequence No.	5'→ 3'	Locus in HPV 32
M3201	TGCTACTGTAACAACTGAAG	6906 – 6925
M3202	GCTACTGTAACAACTGAAGA	6907 – 6926
M3203	TACTGTAACAACTGA	6909 – 6923
M3204	ACTGTAACAACTGAAGACAC	6910 – 6929
M3205	CAACTGAAGACACATACAAGTC	6917 – 6938
M3206	CAAAATTACATTATCTGTAGAGG	7005 – 7027
M3207	TCCTGACATACTAGACGAT	7053 – 7071
M3208	TGTAGCTCCACCGCCCTCTGGTACTT	7083 – 7108
M3209	TTATAGATTTGTGCAGTCTCAG	7119 –7140
M3210	TAAGGTAACAGCACCTGAA	7158 – 7176
M3211	TTTTTCTGACTATTCA	7188 – 7203
M3212	AGCACATGCAGATAAGGAGGCAG	1062 - 1084
M3213	GGCAGTCCATATGAAAGTCCCGCCAGTGAT	1111 - 1140
M3214	GAGCTAAGCCCTAGGCTTGGTGGATT	1162 - 1187
M3215	GGGGCCAAACGACGACTATTTCAATC	1210 - 1235
M3216	GCAGGAACAGGTAGAAAATGGACATG	1284 - 1309
M3217	GTGTACATGGGGTGCAGGAAAATCAG	1352 - 1377
M3218	GCCTACAACAAGGGTGGTGGAATTGC	1395 - 1420
M3219	GCAAGCAACATTGTTAGGTAAGTTTA	1437 - 1462
M3220	GCTGTTTGGATTGTCATTTGGTGATT	1467 - 1492

Table 7. Sequences and loci of oligonucleotides for identifying HPV 33

Sequence No.	5'→ 3'	Locus in HPV 33

M3301	TATGCACACAAGTAACTAGT	6624 – 6643
M3302	CACACAAGTAACTAG	6628 – 6642
M3303	ACAAGTAACTAGTGACAGTA	6631 – 6650
M3304	GTAACTAGTGACAGTACATATAA	6635 – 6657
M3305	GTACATATAAAAATGAAAAT	6648 – 6667
M3306	CAAAGTTACCTTAACTGCAGAAG	6727 – 6749
M3307	TCCAGATATTTTAGAAGAT	6775 – 6793
M3308	TTTAACACCTCCTCCATCTGCTAGTT	6805 – 6830
M3309	CTATAGGTTTGTTACCTCTCAG	6841 – 6862
M3311	AACAGTACCTCCAAAGGAA	6880 – 6898
M3312	CTTAGGTAAATATACA	6910 – 6925
M3313	AGGGGAGGATGATTTAAATGCTG	1100 - 1122
M3314	GCATGTTCACAAAGTGCTGCGGAGGA	1149 - 1174
M3315	GATCGTGCTGCAAACCCGTGTAGAAC	1182 - 1207
M3316	GAATGCACATACAGAAAACGAAAAATAG AT	1227 - 1256
M3317	AAATAGATGAGCTAGAAGACAGCGGA	1249 - 1274
M3318	GGTACAACAGGTAGAAAGTCAAAATG	1307 - 1332
M3319	GACTTAGAATCTAGTGGGGTGGGGGA	1350 - 1357
M3320	CTGTGAGACAAATGTAGATAGCTGTG	1391 - 1416
M3321	CGTTGCAGGAAATTAGTAATGTTCTA	1426 - 1451
M3322	GAGGCCTATGGAATAAGTTTTAT	1494 - 1516

Table 8. Sequences and loci of oligonucleotides for identifying HPV 35

Sequence No.	5'→ 3'	Locus in HPV 35
M3501	TCTGCTGTGTCTTCTAGTGA	6612 – 6631
M3502	TGCTGTGTCTTCTAG	6614 – 6628
M3503	GTGTCTTCTAGTGACAGTAC	6618 – 6637
M3504	CTTCTAGTGACAGTACATATAAA	6622 – 6644
M3505	GTACATATAAAAATGACAAT	6634 – 6653
M3506	TAAAATAACACTAACAGCAGATG	6713 – 6735
M3507	CCCGTCCATTTTAGAGGAT	6761 – 6779
M3508	CCTTACACCACCGCCTTCTGGTACCT	6791 – 6816
M3509	ATATCGCTATGTAACATCACAG	6827 – 6848
M3510	ACCCAGTGCACCAAAACCT	6866 – 6884
M3511	GGAGCAAACACACAAAGAGGCTG	1089 - 1111

M3512	GCAGCGTGAGCTTATGTGTTAATAATAAC A	1151 - 1180
M3513	GTCCACGTTTAAAAGCTATTTGCA	1184 - 1207
M3514	CGATTATTTGAACTACCAGACAGCGG	1237 - 1262
M3515	GAGATACAACAGGTAGAGGGGCATGA	1291 - 1316
M3516	GGGCAGTGGGGATAGTATAACCTCTA	1338 - 1363
M3517	GACATGATGAGACTCCAACGCGAGAC	1376 - 1401
M3518	CTAAAATGTAGTAATGCAAACGCAGC	1414 - 1439
M3519	GCTATGTTGGCTAAATTTAAAGAACT	1438 - 1463

Table 9. Sequences and loci of oligonucleotides for identifying HPV 37

Sequence No.	5'→ 3'	Locus in HPV 37
M3701	TGTCTACTGACAATG	6782 – 6796
M3702	TGTCTACTGACAATGGCGAA	6782 – 6801
M3703	TGACAATGGCGAAGTTACAG	6789 – 6808
M3704	GACAATGGCGAAGTTACAGA	6790 – 6809
M3705	AATGGCGAAGTTACAGAATA	6793 – 6812
M3706	CAGAATATAATTCTCAAACA	6806 – 6825
M3707	TAAAGTTCCTTTAAAGGCTGAGG	6885 – 6907
M3708	TTCTGGTATATTGGAAGAG	6933 – 6951
M3709	ATTTGTACCTACTCCAGATAATTCAG	6963 – 6988
M3710	TTATAGGTACATTAATTCAAAG	6999 – 7020
M3711	TGCAGTTGTTGAAAAAGAA	7038 – 7056
M3712	CTTTGCAAAATATACA	7068 – 7083

Table 10. Sequences and loci of oligonucleotides for identifying HPV 39

Sequence No.	5'→ 3'	Locus in HPV 39
M3901	CTCTATAGAGTCTTC	6677 – 6691
M3902	TAGAGTCTTCCATACCTTCT	6682 – 6701
M3903	ATAGAGTCTTCCATACCTTC	6681 – 6700
M3904	GTCTTCCATACCTTCTACATATG	6686 – 6708
M3905	CTACATATGATCCTTCTAAG	6700 – 6719
M3906	TACTGTCACATTAACAACTGATG	6779 – 6801
M3907	TTCCTCTATATTGGACAA	6827 – 6844
M3908	TGTAGCTCCTCCACCATCTGCCAGTT	6857 – 6882
M3909	TTACAGATACCTACAGTCTGCA	6893 – 6914

M3910	GGATGCTCCAGCACCTGAA	6932 – 6950
M3911	ATATGACGGTCTAAAG	6962 – 6977
M3912	GGCCCAAAGGGATGCACAAGCAG	1149 – 1171
M3913	CAGACAGCAGTGGCGACACTAGACCGTATG	1196 – 1225
M3914	GTAGGCAGGAATACCAGGGGAACAC	1234 – 1258
M3915	GCAGTACGCAGGCAACACAAACGGTG	1283 – 1308
M3916	GGAGGAGGTAACTGTAGCAACTAATA	1362 – 1387
M3917	CATGGCGGCAGTGTACGGGAGGAGTG	1411 – 1436
M3918	GGATAGTGCTATAGATAGTGAAAACC	1446 – 1471
M3919	CTCCAACTGCACAAATTAAATTATTG	1484 – 1509
M3920	CCAATAACAAAAAGGCTGCAATGCTA	1517 – 1542

Table 11. Sequences and loci of oligonucleotides for identifying HPV 44

Sequence No.	5'→ 3'	Locus in HPV 40
M4401	TGCCACTACACAGTC	6719 – 6733
M4402	CTACACAGTCCCCTCCGTCT	6724 – 6743
M4403	TGCCACTACACAGTCCCCTC	6719 – 6738
M4404	CAGTCCCCTCCGTCTACATATA	6729 – 6750
M4405	CTACATATACTAGTGAACAA	6742 – 6761
M4406	TAGTATTACCTTAACGGCGGAGG	6821 – 6843
M4407	TGCTGGTATTTTAGAACAG	6869 – 6887
M4408	GTTGTCGCCGCCCCCAAATGGTACCT	6899 – 6924
M4409	ATACAGATATGTGCAGTCCCAG	6935 – 6956
M4410	GCCACCCCCTGAAAAGGCA	6974 – 6992
M4411	CTATGCAAAATTAAGT	7004 – 7019
M4412	GGCGGATGCTCATTATGCGGCTG	1038 – 1060
M4413	GGTAGTCCATATGTTAGTCCTTTAAGTAAT	1087 – 1116
M4414	CACGGCTGGACGCTATAACATTAAGT	1148 – 1173
M4415	GACGGCTGTTTGACAGACCAGAATTA	1196 – 1221
M4416	GCTGAAACGCAGGTAGAGAGAAATGG	1255 – 1280
M4417	GGGAGGTGGACAAGGAAGGGACACAG	1299 – 1324
M4418	GGAAGTGCAGACACATAGCAACACAC	1347 – 1372
M4419	GGTACTAGAACTATTGAAATGTAAGA	1395 – 1420
M4420	GCTTGGTAAGTTTAAGGATTGCTATG	1437 – 1462

Table 12. Sequences and loci of oligonucleotides for identifying HPV 45

Sequence No.	5'→ 3'	Locus in HPV 45
M4501	TGCCTCTACACAAAATCCTG	6651 – 6670
M4502	CTCTACACAAAATCC	6654 – 6668
M4503	ACAAAATCCTGTGCCAAGTA	6660 – 6679
M4504	CAAAATCCTGTGCCAAGTAC	6661 – 6680
M4505	AATCCTGTGCCAAGTACATATG	6664 – 6685
M4506	GTACATATGACCCTACTAAG	6677 – 6696
M4507	CACTATTACTTTAACTGCAGAGG	6756 – 6778
M4508	TAGTAGTATATTAGAAAAT	6804 – 6822
M4509	TGTCCCTCCACCACCTACTACAAGTT	6834 – 6859
M4510	ATATCGTTTTGTGCAATCAGTT	6870 – 6891
M4511	GGATACTACACCTCCAGAA	6909 – 6927
M4512	AGTTCAGAATGATGCACAGGTGT	1135 – 1157
M4513	GCAGCTAAGTGTGGATACGGATCTAAGTCC	1216 – 1245
M4514	CAAGAAATTTCATTAAATAGTGGGCA	1253 – 1278
M4515	ACGGTTGTTTACAATATCAGATAGTG	1294 – 1319
M4516	CATAGTACACAAAGTAGTGGTGGGGA	1397 – 1422
M4517	GACAATGCAGAAAATGTAGATCCGCA	1430 – 1455
M4518	GGAGCTATTACAAGCAAGTAACAAAA	1477 – 1502
M4519	GCAATGCTGGCAGTATTTAAAGACA	1508 – 1532
M4520	ATATGGGCTGTCATTTACGGATTTGG	1534 – 1559

Table 13. Sequences and loci of oligonucleotides for identifying HPV 51

Sequence No.	5'→ 3'	Locus in HPV 51
M5101	CACTGCCACTGCTGCGGTTT	6555 – 6574
M5102	TGCCACTGCTGCGGT	6558 – 6572
M5103	CACTGCTGCGGTTTCCCCAA	6561 – 6580
M5104	CCACTGCTGCGGTTTCCCCA	6560 – 6579
M5105	CTGCGGTTTCCCCAACATTTAC	6566 – 6587
M5106	CAACATTTACTCCAAGTAAC	6578 – 6597
M5107	TAAAATTACTTTAACTACAGAGG	6657 – 6679
M5108	TCCTACCATTCTTGAACAG	6705 – 6723
M5109	ATTAACATTACCTCCGTCTGCTAGTT	6735 – 6760
M5110	ATATAGGTTTGTTAGAAATGCA	6771 – 6792
M5111	GGACACCCCTCCACAGGCT	6810 – 6828

M5112	TTTGGCCAAATATAAA	6840 – 6855
M5113	ATTACAGGCAAACAAAGAGGCTG	1092 – 1114
M5114	GCGAAGCAGCCCATTAGGAGACATTACAAA	1149 – 1178
M5115	CCATAGTCAGGCAAACGAGTCACAAG	1197 – 1222
M5116	AGATTACTGGACAGTTATCCGGACA	1231 – 1255
M5117	CAGGTAGATGGGCAACATGGCGGTTC	1300 – 1325
M5118	AGCTGTGCAAATGTAGAACTAAACAG	1384 – 1409
M5119	GGTATTAGTTATAATGAGTTGGTACG	1477 – 1502

Table 14. Sequences and loci of oligonucleotides for identifying HPV 52

Sequence No.	5'→ 3'	Locus in HPV 52
M5201	TGAGGTTAAAAAGGA	6695 – 6709
M5202	TGAGGTTAAAAAGGAAAGCA	6695 – 6714
M5203	GAGGTTAAAAAGGAAAGCAC	6696 – 6715
M5204	TTAAAAAGGAAAGCACATAT	6700 – 6719
M5205	AAAGGAAAGCACATATAAAAAT	6704 – 6725
M5206	GCACATATAAAAATGAAAAT	6712 – 6731
M5207	CAAAATTACATTAACAGCTGATG	6791 – 6813
M5208	TGCCACTATTTTAGAGGAC	6839 – 6857
M5209	CCTTACCCCACCACCGTCTGCATCTT	6869 – 6894
M5210	ATACAGATTTGTCACTTCTACT	6905 – 6926
M5211	AAACACACCACCTAAAGGA	6944 – 6962
M5212	TTTAAAGGACTATATG	6974 – 6989
M5213	AGGGGAGGATGATTTACATGCTG	1085 – 1107
M5214	GCAGTCCGGAAAGTGCTGGGCAAGATGGTG	1135 – 1164
M5215	GGTAGTCCGCGTGCAAAACACATTTG	1176 – 1201
M5216	CCAAAACGCAAACCATGTCACGTAGA	1224 – 1249
M5217	CAAAATGGCGACTGGCAAAGTA	1311 – 1332
M5218	GCTAGTAATTCAGATGTAAGTTGTAC	1359 – 1384
M5219	GAGGAAAATAGTAATAGAACGCTAAA	1401 – 1426
M5220	GCGAAAATAGCATAAAAACAACTGTA	1447 – 1472
M5221	AGAAACATATGGTGTTAGCTTTATGG	1487 – 1512

Table 15. Sequences and loci of oligonucleotides for identifying HPV 53

Sequence	5'→ 3'	Locus in

No.		HPV 53
M5301	TCCGCAACCACACAGTCTAT	6681 – 6700
M5302	CCGCAACCACACAGT	6682 – 6696
M5303	CCGCAACCACACAGTCTATG	6682 – 6701
M5304	CACAGTCTATGTCTACATATAA	6691 – 6712
M5305	CTACATATAATTCAAAGCAA	6703 – 6722
M5306	TAAAATATCCCTGTCTGCTGAGG	6782 – 6804
M5307	TTCTACCTTACTGGAAGAC	6830 – 6848
M5308	TTTGTCGCCTCCTGTTGCCACTAGCT	6860 – 6885
M5309	ATACAGATATGTGAAAAGTGCA	6896 – 6917
M5310	GGATCAGCCCCCTCCTGAA	6935 - 6953

Table 16. Sequences and loci of oligonucleotides for identifying HPV 54

Sequence No.	5'→ 3'	Locus in HPV 54
M5401	TACAGCATCCACGCA	6633 – 6647
M5402	CAGCATCCACGCAGGATAGC	6635 – 6654
M5403	ACGCAGGATAGCTTTAATAA	6643 – 6662
M5404	CACGCAGGATAGCTTTAATA	6642 – 6661
M5405	ATAGCTTTAATAATTCTGAC	6650 – 6669
M5406	TACCATAACCCTTACAGCAGATG	6729 – 6751
M5407	TCCCACTATTCTAGAGGAC	6777 – 6795
M5408	TATAACCCCCCCAGCTACAAGTAGTT	6807 – 6832
M5409	ATATAGGTTTGTACAGTCACAG	6843 – 6864
M5410	GAATAATGCCCCTGCAAAGGAA	6882 – 6903
M5411	GCTGCAGGCAGATGTAGAGGCAG	1043 – 1065
M5412	CGTATGTAAGTCCTGTTGCAAACAGCGAAC	1099 – 1128
M5413	CTGTGTAGAAAAGGACCTAA	1130 – 1149
M5414	TATATCCCTAGGACGGCGGTCAGCCA	1166- 1191
M5415	GGTAAATACCGAGGGGACAGATGAAA	1265 – 1290
M5416	GAAACAACTACAGATAGCCTAGGAAG	1323 – 1348
M5417	CGTGTAGCATTGTTTGGTATGTTTAA	1386 – 1411
M5418	TATGGATTAAGTTTTATGGACC	1419 – 1440

Table 17. Sequences and loci of oligonucleotides for identifying HPV 56

Sequence	5'→ 3'	Locus in

No.		HPV 56
M5601	CTGCTACAGAACAGT	6630 – 6644
M5602	GCTACAGAACAGTTAAGTAA	6632 – 6651
M5603	CAGAACAGTTAAGTAAATAT	6636 – 6655
M5604	GAACAGTTAAGTAAATATGATGC	6638 – 6660
M5605	GTAAATATGATGCACGAAAA	6648 – 6667
M5606	CAAAATTACTTTGTCTGCAGAGG	6727 – 6749
M5607	TGCTAACCTACTGGAGGAC	6775 – 6793
M5608	GTTATCCCCGCCAGTGGCCACCAGCC	6805 – 5830
M5609	ATATAGATATGTTAGAAGCACA	6841 – 6862
M5610	GGAACAGCCACCAACAGAA	6880 – 6898

Table 18. Sequences and loci of oligonucleotides for identifying HPV 58

Sequence No.	5'→ 3'	Locus in HPV 58
M5801	ATGCACTGAAGTAACTAAGG	6674 – 6693
M5802	CACTGAAGTAACTAAGGAAG	6677 – 6696
M5803	TGAAGTAACTAAGGA	6680 – 6694
M5804	GAAGTAACTAAGGAAGGTAC	6681 – 6700
M5805	CTAAGGAAGGTACATATAAAAA	6688 – 6709
M5806	ATAAAAATGATAATTTTAAG	6703 – 6722
M5807	CAAAATTACACTAACTGCAGAGA	6776 – 6798
M5808	TTCCAATATTTTGGAGGAC	6824 – 6842
M5809	TTTAACACCTCCTCCGTCTGCCAGTT	6854 – 6879
M5810	ATATAGATTTGTTACCTCCCAG	6890 – 6911
M5811	AACAGCACCCCCTAAAGAA	6929 – 6947
M5812	AGGGGTGGACGATATAAATGCTG	1104 – 1126
M5813	GCATGCTCAGAAAGTGCTGTAGA	1153 – 1175
M5814	GCAAATGTGTGTGTATCGTGGAAATATAA A	1195 – 1224
M5815	AAATTATTGAGCTAGAAGACAG	1253 – 1274
M5816	GCACACCAGGTAGAAAGCCAA	1312 – 1332
M5817	GGCTAGTTCAGATGTAAGCAGTGAAA	1377 – 1402
M5818	GTAATATTCTACATAACAGTAA	1445 – 1466
M5819	GCAACGCTATTATATAAATTC	1474 – 1494
M5820	GCTTATGGAGTAAGTTTTATGGAA	1501 – 1524

Table 19. Sequences and loci of oligonucleotides for identifying HPV 59

Sequence No.	5'→ 3'	Locus in HPV 59
M5901	TTCTACTACTTCTTC	6643 – 6657
M5902	ACTACTTCTTCTATTCCTAA	6647 – 6666
M5903	ACTTCTTCTATTCCTAATGT	6650 – 6669
M5904	TCTTCTATTCCTAATGTATACAC	6653 – 6675
M5905	ATGTATACACACCTACCAGT	6666 – 6685
M5906	TAAAATAACATTAACTACAGAGG	6745 – 6767
M5907	TACCACTATTTTGGAGGAT	6793 – 6811
M5908	TGTTACACCACCTCCTACTGCTAGTT	6823 – 6848
M5909	ATACCGTTTTGTTCAATCTGCT	6859 – 6880
M5910	GGACACCGCACCGCCAGTT	6898 – 6916
M5911	TTATGACAAACTAAAG	6928 - 6943
M5912	AGCCCAAAGGGATGCACGGGAAA	1093 – 1115
M5913	GCAGTATAGAAAACAGTAGTGAGAAAGC GG	1143 – 1172
M5914	CCATTACAAGAAATATCAGTAAATG	1196 – 1220
M5915	GGTTAATAACAGTGCCAGACAGCG	1245 – 1268
M5916	GGTAACCGTGGAGAATACTGGAAATG	1306 – 1331
M5917	CTGTAGCGACAGCAGTAACATGGATG	1372 – 1397
M5918	CCCACTAATCAATTGTTACAGTTA	1421 – 1444
M5919	GGGTTATCATTTCAAGATTTGG	1499 - 1520

Table 20. Sequences and loci of oligonucleotides for identifying HPV 61

Sequence No.	5'→ 3'	Locus in HPV 61
M6101	CTGCTACATCCCCCC	6803 – 6817
M6102	ACATCCCCCCCTGTATCTGA	6808 – 6827
M6103	CATCCCCCCCTGTATCTGAA	6809 – 6828
M6104	CCCCTGTATCTGAATATAAAGC	6815 – 6836
M6105	CTGAATATAAAGCCACAAGC	6824 – 6843
M6106	TAAAATACATTTAACCCCTGAAA	6903 – 6925
M6107	TAAGGCCTTGTTGGATGAC	6951 – 6969
M6108	TGTGGTACCACCACCCTCTACCAGTT	6981 – 7006
M6109	ATATAGGTTTTTGCAGTCCAGA	7017 – 7038
M6110	GGGTGCTGCTGCCCCGCCGCCC	7056 – 7077
M6111	CTATGCCAAGTTATCC	7089 – 7104
M6112	TGCACAGGATGACGCTGCAACGG	1038 – 1060

M6113	CCTTGGTGGACAGTGAATTAAGTCCC	1115 – 1140
M6114	GGACAGGACAGGGCTAGGAGAAGGCTGTTT	1168 – 1197
M6115	TGTTTGAGCAAGATAGTGGC	1193 – 1212
M6116	GGATGCGCAACATGAGGGGGGGGGGG	1263 – 1288
M6117	GGCCGAGGCCACAGGTAACCAGGAAA	1335 – 1360
M6118	GGCAGACATATTAGAGGTGTTTAAGG	1380 – 1405
M6119	CTGTACAAATTCAAGGACCTATTTGG	1429 – 1454
M6120	CTAGCATTTGGGGAGCTGGTA	1456 – 1476

Table 21. Sequences and loci of oligonucleotides for identifying HPV 62

Sequence No.	5'→ 3'	Locus in HPV 62
M6201	CCGCCTCCACTGCTG	92 – 106
M6202	GCCTCCACTGCTGCAGCAGA	94 – 113
M6203	CTGCTGCAGCAGAATACACG	101 – 120
M6204	GCAGAATACACGGCTACCAA	109 – 128
M6205	CAGAATACACGGCTACCAAC	110 – 129
M6206	CAAAATACAGTTAACCCCCGAAA	189 – 211
M6207	CAAGGACCTTTTGGATGAC	237 – 255
M6208	GGTTTTACCTCCCCCTTCCACTAGTT	267 – 292
M6209	ATATCACTATTTCGAGTCTCGG	303 – 324
M6210	GGGGCTGCCTACCCGTCCC	342 – 360
M6211	GTATGCGCAAATGACA	372 – 387

Table 22. Sequences and loci of oligonucleotides for identifying HPV 66

Sequence No.	5'→ 3'	Locus in HPV 66
M6601	CAGCTAAAAGCACAT	6680 – 6694
M6602	CAGCTAAAAGCACATTAACT	6680 – 6699
M6603	CTAAAAGCACATTAACTAAA	6683 – 6702
M6604	TTAACTAAATATGATGCCCG	6694 – 6713
M6605	CTAAATATGATGCCCGTGAA	6698 – 6717
M6606	TAAAATAACCTTAACTGCAGAAG	6777 – 6799
M6607	TAATACTTTATTAGACGAT	6825 – 6843
M6608	CTTATCCCCACCAGTTGCAACTAGCT	6855 – 6880
M6609	ATATAGGTATATTAAAAGCACA	6891 – 6912
M6610	GGAACAGCCCCCTGCAGAA	6930 – 6948

M6611	CCTGGCTAAATATAAG	6960 – 6975
M6612	AGCACATGCAGATGCACAGACG	1116 – 1137
M6613	GGTAGTCCCTTAAGTGATATTAGTAA	1165 – 1190
M6614	GCAAACTGTGTACCGAGAGGAAGTAA	1194 – 1219
M6615	AAGGCTAATATTATCAGAAGACAGCGGGT A	1224 – 1253
M6616	GAAACATCACAACAGGTAGAATACG	1276 – 1300
M6617	GAGCTCACAAAATGGAGGCTCGCAAA	1320 – 1345
M6618	ATCAAATATGGATATAGATACAAATA	1371 – 1396
M6619	CCAATTGCAGGAACTATTTAAAAGTA	1413 – 1438
M6620	CAAGGAAGATTACATTTTAAATTTAA	1447 - 1472
M6621	AGAAGTGTATGGAGTGCCAT	1473 – 1492

Table 23. Sequences and loci of oligonucleotides for identifying HPV 67

Sequence No.	5'→ 3'	Locus in HPV 67
M6701	CTGAGGAAAAATCAG	6655 – 6669
M6702	GAGGAAAAATCAGAGGCTAC	6657 – 6676
M6703	ATCAGAGGCTACATACAAAAATG	6665 – 6687
M6704	AGGAAAAATCAGAGGCTACA	6658 - 6677
M6705	CTACATACAAAAATGAAAAC	6673 – 6692
M6706	CAAAATATCCCTTACTGCAAATG	6752 – 6774
M6707	TCCAGATATATTAGAGGAC	6800 – 6818
M6708	CCTTACACCACCTCCTTCAGGTAATT	6830 – 6855
M6709	ATATAGATTTGTTACCTCGCAG	6866 – 6887
M6710	AACATCCCCTCCAACAGCA	6905 – 6923
M6711	TCTTAAAAAGTACAGT	6935 – 6950
M6712	AGAGGAGGATGACCTAAACGCTG	1096 – 1118
M6713	CAGGCATGTGGTGGTAATAGTAATGG	1151 – 1176
M6714	GCCGCAAAACGCAGAGCATACGACATAG AA	1199 – 1228
M6715	GGCAAAATGGCGATATGCAGTGCAGT	1287 – 1312
M6715	GCAAGTAGTACGGGAAACAGTGTAGA	1331 – 1356
M6716	GTCAGGAACAAAGCATGCCATTGCAA	1380 – 1405
M6717	GCATGTAAATAATATAAAGGCAACG	1423 – 1447
M6718	GGAAGCATATGGGGTAACGTTTACAC	1465 – 1490

Table 24. Sequences and loci of oligonucleotides for identifying HPV 68

Sequence No.	5'→ 3'	Locus in HPV 68
M6801	CTACTACTGAATCAG	2653 – 2667
M6802	TGAATCAGCTGTACCAAATA	2660 – 2679
M6803	GAATCAGCTGTACCAAATAT	2661 – 2680
M6804	CAGCTGTACCAAATATTTATGA	2665 – 2686
M6805	ATATTTATGATCCTAATAAA	2677 – 2696
M6806	TCCTGCTATTTTGGATGAT	2804 – 2822
M6807	TACTATAACATTGTCCACTGATG	2756 – 2778
M6808	TGTTGCCCCTCCACCATCTGCTAGTC	2834 – 2859
M6809	ATACCGCTATCTGCAATCAGCA	2870 – 2891
M6810	AGACGCCCCTGCACCTACT	2909 – 2927
M6811	ATATGATGGCTTAAAC	2939 – 2954
M6812	GGCCCAAAGGGATGCACAAACAG	4990 – 5012
M6813	GCCCTTTAGCAAAGTCGCCATTACAG	5055 – 5080
M6814	GGTAACTGTAGCAACTAATA	5115 – 5134
M6815	GGAAAATGGCGACAGCATACGGGAGGACTG	5163 – 5192
M6816	GACAGTGCTATAGATAGTGAAAACCA	5203 – 5228
M6817	CCTACTACGCAACTAAAAGTATTA	5242 – 5265
M6818	AAGCTGCAATGTTAACAGAATTTAAA	5285 – 5310

Table 25. Sequences and loci of oligonucleotides for identifying HPV 69

Sequence No.	5'→ 3'	Locus in HPV 69
M6901	TATTAGTACTGTATCTGCAC	6572 – 6591
M6902	CTGTATCTGCACAAT	6580 – 6594
M6903	CTGTATCTGCACAATCTGCA	6580 – 6599
M6904	TGCACAATCTGCATCTGCCA	6587 – 6606
M6905	CAATCTGCATCTGCCACTTTTA	6591 – 6612
M6906	CCACTTTTAAACCATCAGAT	6604 – 6623
M6907	TAAAATTACTCTTACCACTGATG	6683 – 6705
M6908	TTCTACTATTTTGGAAAAT	6731 – 6749
M6909	CCTTACCTTGCCTCCTACTGCTAGTT	6761 – 6786
M6910	ATATAGGTTTATTAAAAATTCA	6797 – 6818
M6911	CGATGCCCCTGCACAGCCC	6836 – 6854
M6912	AACACAAGCAAATAAGAAGGCAG	1101 – 1123
M6913	GAACAGCCCGTTGCAAGACATAACAA	1158 – 1183

M6914	CAGACGAAGTAAACAATTTACAGGC	1208 – 1232
M6915	GGAGAGCAGTGGACAGTGTTCCGGACAG CG	1238 – 1267
M6916	GGTAGATAAACACAATGAACAAAATG	1308 – 1333
M6917	TCAAGTGGATCTGTATCAGACA	1360 – 1381
M6918	GCACAGGCAAGTAGTGTAACCAAAA	1399 – 1423
M6919	GTAATGTAAAAGCAGCATTATTAA	1445 – 1468
M6920	CAGTATATGGTGTAAGTTATA	1481 – 1501

Table 26. Sequences and loci of oligonucleotides for identifying HPV 6

Sequence No.	5'→ 3'	Locus in HPV 6
M0601	CATCCGTAACTACATCTTCC	6814 – 6833
M0602	ATCCGTAACTACATCTTCCA	6815 – 6834
M0603	CTACATCTTCCACATACACCAA	6823 – 6844
M0604	CATCTTCCACATACACCAAT	6826 – 6845
M0605	ATCTTCCACATACACCAATT	6827 – 6846
M0606	CCACATACACCAATTCTGAT	6832 – 6851
M0607	TAGCATTACATTGTCTGCTGAAG	6911 – 6933
M0608	TCCCTCTGTTTTGGAAGAC	6959 – 6977
M0609	GTTATCGCCTCCCCCAAATGGTACAT	6989 – 7014
M0610	CTATAGGTATGTGCAGTCACAG	7025 – 7046
M0611	GCCCACTCCTGAAAAGGAA	7064 – 7082
M0612	CTATAAGAACCTTAGT	7094 – 7109
M0613	GGCGGACACCCATTATGCGACTG	1045 – 1067
M0614	GTTAGTCCTATAAACACTATAGCCGA	1106 – 1131
M0615	CCACGATTGGACGCCATTAAACTTACAAG A	1154 – 1183
M0616	GGCTGTTTCAAACCAGGGAACTAACG	1206 – 1231
M0617	GGTAGAGAAACATGGCGTACCGGAAA	1279 – 1304
M0618	GGACACAGGAAGGGACATAGAGGGGG	1327 – 1352
M0619	CACAAACAGTGTACGGGAGCATGCAG	1378 – 1403
M0620	GCTAAAATGTAAAGATTTACGGGCAG	1426 – 1451
M0621	GCTTTGGGCTGTCTTTTATAGATTTA	1476 – 1501

Table 27. Sequences and loci of oligonucleotides for identifying HPV 70

Sequence No.	5'→ 3'	Locus in HPV 70

M7001	TGTCTGCCTGCACCGAAACG	6614 – 6633
M7002	CTGCACCGAAACGGC	6621 – 6635
M7003	GAAACGGCCATACCTGCTGT	6628 – 6647
M7004	CGAAACGGCCATACCTGCTG	6627 – 6646
M7005	CGGCCATACCTGCTGTATATAG	6632 – 6653
M7006	CTGTATATAGCCCTACAAAG	6644 – 6663
M7007	TACTATCACATTAACTGCTGACG	6723 – 6745
M7008	TCCTGCAATTTTGGACAAT	6771 – 6789
M7009	AGTTACCCCTCCACCATCTGCAAGCT	6801 – 6826
M7010	GTATAGGTATTTACAATCAGCA	6837 – 6858
M7011	GGATGCTCCTACACCTGAA	6876 – 6894
M7012	CTATGACGATTTAAAA	6906 – 6921
M7013	GGCCCAAAGGGATGCACAATCAG	1149 – 1171
M7014	GCAATCTAAATAAAAGTCCTTGT	1202 – 1224
M7015	GTACATAGGGAACAAAGGGTAACAC	1240 – 1264
M7016	GGTAAACATATGCAATAAACAGG	1278 – 1300
M7017	ACAAACGTGTATTCAGTACCAGACAGCGGC	1306 – 1335
M7018	GTAGTAAATAATACAAATGGGGAAGA	1378 – 1403
M7019	GGAGTGCAGTAGTGTAGACAGTGCTA	1446 – 1471
M7020	TCCACAGTCACCTACTGCACAGCTAA	1491 – 1516
M7021	GCTAATAACCAAAAAGCCATACTAC	1531 – 1555
M7022	CACACATATGGATTAGCATTTAACGA	1570 – 1595

Table 28. Sequences and loci of oligonucleotides for identifying HPV 72

Sequence No.	5'→ 3'	Locus in HPV 72
M7201	ATCTGTTGGTTTAATGAGCT	6759 – 6778
M7202	TTTGTGACAGTTGTAGATAC	6780 – 6799
M7203	CTGCCACAGCGTCCT	6829 - 6843
M7204	ACAGCGTCCTCTGTATCAGA	6834 – 6853
M7205	CCACAGCGTCCTCTGTATCA	6832 – 6851
M7206	AGCGTCCTCTGTATCAGAATAT	6836 – 6857
M7207	CAGAATATACAGCTTCTAAT	6850 – 6869
M7208	TAAAATTCACTTAACTCCTGAAA	6929 – 6951
M7209	TAAGGCCTTATTGGATGAC	6977 – 6995
M7210	TGTGGTGCCTCCTCCTTCTACCAGTT	7007 – 7032
M7211	CTATAGGTTTTTGCAGTCTCGT	7043 - 7064

M7212	GGGGGCTGCCACCCCTCCTCCT	7082 – 7103
M7213	ATATGCTAACTTATCC	7115 – 7130

Table 29. Sequences and loci of oligonucleotides for identifying HPV 74

Sequence No.	5'→ 3'	Locus in HPV 74
M7401	CCTACCTCACAATCG	1686 – 1700
M7402	CTCACAATCGCCTTCTGCTA	1691 – 1710
M7403	ACCTCACAATCGCCTTCTGC	1689 – 1708
M7404	CAATCGCCTTCTGCTACATATA	1695 – 1716
M7405	ACAATCGCCTTCTGCTACATAT	1694 - 1715
M7406	CTACATATAATAGTTCAGAC	1708 – 1727
M7407	TAGTATTAAGTTAACTGCTGAGG	1787 – 1809
M7408	TCCTACAGTTTTAGAAGAG	1835 –1853
M7409	GCTAACGCCTCCCCCCAATGGTACTT	1865 – 1890
M7410	CTACAGATATGTGCAGTCCCAG	1901 – 1922
M7411	ACCTACGCCTGATAAAGCA	1940 – 1958
M7412	CTATGCAAATTTAAGT	1970 – 1985

Table 30. Sequences and loci of oligonucleotides for identifying HPV 82

Sequence No.	5'→ 3'	Locus in HPV 82
M8201	TGCTGTTACTCCATC	6608 – 6622
M8202	TGCTGTTACTCCATCTGTTG	6608 – 6627
M8203	ACTCCATCTGTTGCACAAAC	6615 – 6634
M8204	AAACATTTACTCCAGCAAAC	6631 – 6650
M8205	TAAAATCACTTTAACTACTGAAA	6710 – 6732
M8206	TTCTACAATTTTAGAACAG	6758 – 6776
M8207	ATTAACATTGCCCCCCTCCGCTAGTT	6788 – 6813
M8208	CTATCGATTTGTAAAAAATGCA	6824 – 6845
M8209	GGACAGTCCTCCACAGGCT	6863 – 6881
M8210	AACACAGGCACACAAAGAGGCTG	1094 – 1116
M8211	GCAGCCCATTAAAAGACATTACAAA	1156 – 1180
M8212	GTCAGCAACAACCAAAACAGGCAAACCTTC	1201 – 1230
M8213	GGAGATTACTGGACAGTTATCCGGACA	1243 – 1269
M8214	CCTTACAGGTAGATGGGCAAAATGAC	1309 – 1334
M8215	GAGCAGCGACAGAAGTACAGAGATAG	1367 – 1392

M8216	GCTACCAATGTAGGACTAAACAGTA	1413 – 1437
M8217	GTAGCAATGCAAAAGCAATGTTTATG	1456 – 1481
M8218	GGTGTTAGTTATAATGAGTTGGTAAG	1503 – 1528

Table 31. Sequences and loci of oligonucleotides for identifying HPV CP8061

Sequence No.	5'→ 3'	Locus in HPV CP8061
M806101	TCTGTGCTACCAAAACTGTT	86 – 105
M806102	CTACCAAAACTGTTG	92 – 106
M806103	ACCAAAACTGTTGAGTCTAC	94 – 113
M806104	AACTGTTGAGTCTACATATAAA	99 – 120
M806105	GTTGAGTCTACATATAAAGC	103 – 122
M806106	CTACATATAAAGCCTCTAGT	110 – 129
M806107	TGTTATTAATTTAACAGCTGAAA	189 – 211
M806108	TGCTACATTACTGGAGGAC	237 – 255
M806109	GTTCTTACCACCTCCTACTG	267 – 286
M806110	CTACCGCTTTTTACAGTCTCAG	303 – 324
M806111	AAACAGTCCTCCTCCTGCAGAA	342 – 363
M806112	CTATGCAGATCTTACA	375 – 390

Table 32. Sequences and loci of oligonucleotides for identifying HPV CP8034

Sequence No.	5'→ 3'	Locus in HPV CP8034
M830401	CAGCTACATCTGCTG	92 – 106
M830402	GCTACATCTGCTGCTGCAGA	94 – 113
M830403	ACATCTGCTGCTGCAGAATACA	97 – 118
M830404	TGCTGCAGAATACAAGGCCT	105 – 124
M830405	GCTGCAGAATACAAGGCCTC	106 – 125
M830406	CAGAATACAAGGCCTCTAAC	110 – 129
M830407	TAAAATACAGTTAACACCAGAAA	189 - 211
M830408	CAAGGCACTGTTGGATGAT	237 – 255
M830409	TGTGTTGCCACCTCCTTCCACCAGTT	267 – 292
M830410	ATATCGCTTTTTACAGTCTCGG	303 – 324

M830411	GGGTGCTGCTGCCCCTGCGCCC	342 – 363
M830412	TTATGCCGACATGTCA	375 – 390

Table 33. Sequences and loci of oligonucleotides for identifying HPV L1AE5

Sequence No.	5'→ 3'	Locus in HPV L1AE5
MAE501	ATCTACTGCAACTACTAATC	69 – 88
MAE502	CTGCAACTACTAATC	74 – 88
MAE503	CTGCAACTACTAATCCAGTT	74 – 93
MAE504	ACTACTAATCCAGTTCCATCTA	79 – 100
MAE505	CTAATCCAGTTCCATCTATA	83 – 102
MAE506	CTATATATGAACCTTCTAAA	98 – 117
MAE507	TAAAATTACACTTACTACTGATG	177 – 199
MAE508	TCCTACTATTTTAGATAGT	225 – 243
MAE509	TGTTAGTCCTCCCCCATCTGCTAGCT	255 – 280
MAE510	ATATAGGTTTTTACAGTCATCT	291 – 312
MAE511	GGATGTGGTTGTTCCACAA	330 – 348

Table 34. Sequences and loci of oligonucleotides for identifying HPV MM4

Sequence No.	5'→ 3'	Locus in HPV MM4
MM401	CTGCTGTTACTCAATCTGTT	92 – 111
MM402	TGCTGTTACTCAATC	93 – 107
MM403	GTTACTCAATCTGTTGCACA	97 – 116
MM404	TGCACAAACATTTACTCCAG	111 – 130
MM405	TTACTCAATCTGTTGCACAAAC	98 – 119
MM406	AAACATTTACTCCAGCAAAC	116 – 135
MM407	TAAAATCACTTTAACTACTGAAA	195 – 217
MM408	TTCTACAATTTTAGAACAG	243 – 261
MM409	ATTAACCTTGCCCCCCTCAGCTAGTT	273 – 298
MM410	CTATCGATTTGTAAAAAATGCA	309 – 330
MM411	GGACAGTCCTCCACAGGCT	348 – 366

Table 35. Sequences and loci of oligonucleotides for identifying

HPV MM7

Sequence No.	5'→ 3'	Locus in HPV MM7
MM701	TGCTGCTACACAGGC	93 – 107
MM702	GCTGCTACACAGGCTAATGA	94 – 113
MM703	TGCTACACAGGCTAATGAAT	96 – 115
MM704	CTACACAGGCTAATGAATACAC	98 – 119
MM705	ATGAATACACAGCCTCTAAC	110 – 129
MM706	CAAAATACATCTTACCCCTGAAA	189 – 211
MM707	TGAACATTTATTGGATGAG	237 – 255
MM708	CGTGTTACCACCTCCTTCCACCAGCC	267 – 292
MM709	CTATCGCTATCTGCAGTCCCGT	303 – 324
MM710	GGGTCCTTCCGCCCCTGCCCCT	342 – 363
MM711	TTATGATGGCCTTGTA	375 – 390

Table 36. Sequences and loci of oligonucleotides for identifying

HPV MM8

Sequence No.	5'→ 3'	Locus in HPV MM8
MM801	TGCTACCAACACCGA	93 –107
MM802	CTACCAACACCGAATCAGAA	95 –114
MM803	CCAACACCGAATCAGAATATAA	98 –119
MM804	CAGAATATAAACCTACCAAT	110 – 129
MM805	TAAGGTCCGTCTGACTCCAGAGG	189 – 211
MM806	TGACTCCTTATTAGATGAG	237 – 255
MM807	TGTTGTGCCCCCTCCCTCCACAAGTT	267 – 292
MM808	CTATAGGTACTTGCAGTCTCGC	303 – 324
MM809	GGGGGCCGCCGCCGCCAAGCCT	342 – 363
MM810	TTATGCTGGCATGTCC	375 – 390

Table 37. Sequences and loci of oligonucleotides for identifying

HPV 42

Sequence No.	5'→ 3'	Locus in HPV 42
M4201	TATATGTTGGGGAAATCAGCTA	6802 - 6823
M4202	CACTGCAACATCTGGTGATA	6874 - 6893
M4203	GCAACATCTGGTGATACATATACAGCTG	6878 - 6907

	CT	
M4204	CATTAACTGTTGAAGTTATGTCA	6978 - 7000
M4205	CCTAACATATTAGAGGAGTGGAATGT	7019 - 7044
M4206	CACCACCACCTTCAGGAACT	7053 - 7072
M4207	GTTATAGGTATGTACAATCAGAAG	7083 - 7106
M4208	GCTAAGGTAACAACGCCAGAAAAAAAGG AT	7121 - 7150
M4209	CAGACTTTTGGTTTTGGGAGGTAA	7158 - 7181
M4210	GAAAAGTTTTCTACTGATTTA	7190 - 7210
M4211	GTCAGTGATTCACAGCACAGCATAG	1111 – 1135
M4212	GTCCTAGGCTTGGCGGTTTAA	1148 - 1168
M4213	CCCAAGGGGCCAAACGACGATTATTCCA GT	1184 - 1213
M4214	CAGCAGACACAGGTAGAACACGGACA	1261 - 1286
M4215	GCAGTGGGTAGTGAACTTGGGG	1321 - 1342
M4216	GAAGAAGGTAGTACTACAAGTACGCC	1354 – 1379
M4217	GGTAGAATTACTTAAGTGTAAGAAC	1392 - 1416
M4218	GTTAGGTAAGTTTAAAGAATTG	1431 - 1452
M4219	GTCATTTGGCGATTTAGTAAGA	1461 - 1482

Table 38. Sequences and loci of oligonucleotides for identifying HPV 43

Sequence No.	5'→ 3'	Locus in HPV 43
M4301	CATTTGTTTTGGGAATCAGTTG	21 - 42
M4302	TGACCCTACTGTGCCCAGTA	99 - 118
M4303	ACTGTGCCCAGTACATATGACAATGCAA AG	106 - 135
M4304	GTTTATATTTCAATTATGCATAA	177 - 199
M4305	CCAGAGGTTATGACATATATT	211 – 231
M4306	CCCACATTATTAGAGGACTGGAA	244 - 266
M4307	CCACCTGCCTCTGCTTCTTTG	280 - 300
M4308	CGCTTTTTGTCTAACAAGGCCATTG	313 – 337
M4309	CCAAAGGAACGGGAGGATCCCTA	358 - 380
M4310	CTTACAGAAAAGTTTTCTGCACAAC	409 - 433

Each dot on the carrier 11 is an oligonucleotide selected from Tables 1 to 36. For example, an oligonucleotide on the carrier 11 could be

selected from one of the sequences numbered M1101 to M1124 (shown in Table 1) for indentifying the subtype 11 of human papilloma viruses (HPV 11).

The method for mounting the oligonucleotides on the carrier 11 (the nylon membrane) is described as follows.

1.-TTTTTTTTTTTTTTT is added to the 3' end of the oligonucleotide provided by the present invention by terminal transferase according to the following steps 1.1 to 1.3.

1.1 Mixing the following components:

10X NEBuffer 4	5 μl
2.5 mM $CoCl_2$	5 μl
oligonucleotide	5 ~ 300 pmol
10 ~ 300 mM dATP、dCTP、dTTP or dGTP	1 μl
Terminal Transferase (20U/ul) (NEW English BioLabs,M0252S)	0.5 ~ 5 μl
Add M.Q. H_2O to final volume	50 μl

1.2 The components are mixed at 37℃ for 15~60 minutes.

1.3 10 μl of 0.2 M EDTA (pH 8.0) is added to the mixture to stop the reaction.

2. The oligonucleotide having 3' end labeling is mounted on the carrier 11 according to the following steps 2.1 to 2.3.

2.1 The oligonucleotide having 3' end labeling is mounted on the carrier 11 by a needle having a 400 μm wide head. The distance between each dot is 1200 μm.

2.2 The carrier 11 having the dot array 12 thereon is exposed to UV light, and the detector 10 is formed.

2.3 The detector 10 is preserved in a drying box.

Please refer to Fig. 2. A detector 20 is the second embodiment of the present invention for simultaneously detecting and identifying subtypes of human papilloma viruses contained in a sample. The detector 20 includes a carrier 21 and a dot array 22. The carrier 21 is a glass plate having the dot array 22 mounted thereon. Each dot in the dot array 12 is an oligonucleotide (15~30mer) for identifying a specific subtype of human papilloma viruses.

Each dot on the carrier 21 is an oligonucleotide selected from Tables 1 to 36. For example, an oligonucleotide on the carrier 21 could be selected from one of the sequences numbered M1101 to M1124 (shown in Table 1) for indentifying the subtype 11 of human papilloma viruses (HPV 11).

The method for mounting the oligonucleotides on the carrier 21 (the glass plate) is described as follows.

1. The surface of the carrier 21 is treated according to the following steps 1.1 to 1.8.

1.1 The carrier 21 is cleaned in non-fluorescent and soft cleaner.

1.2 The clean carrier 21 is immersed in 10% NaOH.

1.3 The carrier 21 is oscillated in double-distilled water, 1% HCl solution and methanol in sequence for 2 minutes, and dried in an oven.

1.4 The carrier 21 is immersed in 1% 3-aminopropyltrimethoxysilane (APTMS) in 95% aqueous acetone at room temperature for about 2 minutes.

1.5 The carrier 21 is washed in acetone, and the carrier 21 is dried in the oven at 110℃ for 45 minutes.

1.6 The dried carrier 21 is immersed in 0.2% 1,4-phenylene diisothiocyanate, wherein the solvent is 10% pyridine in dimethyl formamide), at room temperature for 2 hours.

1.7 The carrier 21 is washed in methanol and acetone, and then the carrier 21 is dried.

1.8 The dried carrier 21 is preserved in a vacuum and dry box.

2. The oligonucleotides provided by the present invention are mounted on the carrier 21 (the glass plate) according to the following steps 2.1 to 2.3.

2.1 The oligonucleotide having 3' end labeling is mounted on the carrier 21 by a needle having a 400 μm wide head. The distance between each dot is 1200 μm.

2.2 The carrier 21 is immersed in 1% NH_4OH solution for about 2 minutes, washed in double-distilled water, and then dried at room temperature. Thus, the detector 20 is formed.

2.3 The detector 20 is preserved in a dried box.

The method provided by the present invention for simultaneously detecting and identifying subtypes of human papilloma viruses contained in a sample is described as follows.

1. The sample is treated according to the following steps 1.1 to 1.3.

1.1 The cells are centrifuged at 1,500 rpm at 20℃ for 5 minutes.

1.2 The cell pellet is washed in 10 mM Tris (pH 8.5) and dissolved in 8 mM NaOH. Then, the solution is transfer to 1.5 mL micro-tube.

1.3 A proper amount of TreTaq (1U/μl) solution is added to the micro-tube. The reaction is carried out at 95℃ for 1 hour. The DNA contained in the sample is obtained after centrifugation at 13,500 rpm, 20℃ for 5 minutes. The otained DNA is preserved at -20℃.

2 Polymerase chain reactions are performed according to the following steps.

2.1 Glutaldehyde-3-phosphodehydrogenase gene is used as the internal control of the polymerase chain reactions according to the following steps 2.1.1 to 2.1.3.

2.1.1 Mixing the following components:

Reagent	Stock	amount	Final concentration
Sterile H_2O		2.6	
10X *Taq* Buffer		0.5	1X *Taq* Buffer
dNTP	2.5 mM	0.4	200 μM
Template		1	
GAP241-5[1)] primer	10 pmol/μl	0.2	0.4 pmol/μl
GAP241-3[2)] primer	10 pmol/μl	0.2	0.4 pmol/μl
ProTaq (PROTECH)	5 U/μl	0.1	0.1 U/μl
Total volume (μl)		5	

1) Gap21-5 : CCACCAACTGCTTAGCACCCC

2) Gap21-3 : TGCAGCGTACTCCCCACATCA

3) The proper amount of mineral oil is added to prevent the evaporation.

2.1.2 The polymerase chain reaction is performed according to the following programs.

Program 1	Program 2	Program 3

	94℃，15 秒	
94℃，	57℃，	72℃，
3 minutes	1 minute	5 minutes
	72℃，30 seconds	
	40 cycles	

2.1.3 The product of the polymerase chain reaction is analyzed in 2.5% agarose/EtBr (0.5×TBE).

2.2 The DNA contained in the sample is proceeded the first round of polymerase chain reaction according to the following steps.

2.2.1 Mixing the following components:

Reagent	Stock	Amount	Final concentration
Sterile H_2O		4.7-5.7	
10X *Taq* Buffer		1	1X *Taq* Buffer
dNTP	2.5 mM	0.8	200 μM
Template		1-2	
BSA	10 mg/ml	0.1	0.1 μg/μl
Primer[1,2)]	10 pmol/μl	0.6	0.6 pmol/μl
Primer[1,2)]	10 pmol/μl	0.6	0.6 pmol/μl
ProTaq (PRO_{TECH})	5 U/μl	0.2	0.1 U/μl
Total volume (μl)		10	

1) MY09/MY11：Manos *et al.*, *Cancer Cells* 1989, 7, 209-214；

2) E1 301L/E1 847R：*Ylitalo et al., J. Clin. Microbiol.* 1995, 33,

1822-1828；

3) The proper amount of mineral oil is added to prevent the evaporation.

2.2.2 The polymerase chain reaction is performed according to the following programs.

Program 1	Program 2	Program 3
	94℃，45秒	
94℃，	45℃，	72℃，
3 minutes	1 minute	5 minutes
	72℃，	
	1.5 minutes	
	45 cycles	

2.2.3 The product of the polymerase chain reaction is analyzed in 2.5% agarose/EtBr (0.5×TBE).

2.3 The DNA contained in the sample is proceeded the second round of polymerase chain reaction via internal primers according to the following steps.

2.3.1 Mixing the following components:

Reagent	Stock	Amount	Final concentration
Sterile H_2O		11.75	
10X *Taq* Buffer		2.5	1X *Taq* Buffer
dNTP	2.5 mM	2	200 μM
First round PCR product		5	
BSA	10 mg/ml	0.25	0.1 μg/μl

Primer[1)]	10 pmol/μl	1.5	0.6 pmol/μl
Primer[1,2,3)]	10 pmol/μl	1.5	0.6 pmol/μl
ProTaq (PRO_{TECH})	5 U/μl	0.5	0.1 U/μl
Final volumn (μl)		25	

1) GP5+/GP6+ : De Roda Husman *et al., J. Gen. Virol.* 1995, 76, 1057-1062 ;

2) E1 350L/E1 547R : Ylitalo *et al. J. Clin. Microbiol.* 1995, 33, 1822-1828 ;

3) The 5' end of the GP6+ and E1 547R primers could be labeled with biotin or Cy5 fluorescent substances.

4)The proper amount of mineral oil is added to present the evaporation.

2.3.2 The polymerase chain reaction is performed according to the following programs.

Program 1	Program 2	Program 3
	94℃ , 45 seconds	
94℃ , 3 minutes	45℃ , 1 minute	72℃ , 5 minutes
	72℃ , 1.5 minutes	
	45 cycles	

2.3.3 The product of the polymerase chain reaction is analyzed in 2.5% agarose/EtBr (0.5×TBE).

3. The detector 10 provided by the present invention is used for

identifying the subtypes of human papilloma viruses according to the following steps.

3.1 The detector 10 is immersed in 2x SSC solution for 5 minutes.

3.2 The detector 10 is immersed in a buffer containing salmon sperm DNA (50 μg/μl), and the oligonucleotides mounted on the detector 10 are pre-hybridized with the salmon sperm DNA at 35℃ for 30 minutes.

3.3 The PCR product having biotin labeled thereon is added into and mixed with a buffer containing salmon sperm DNA (50 μg/μl) at 95℃ for about 5 minutes. The denatured DNA is placed on ice.

3.4 The denature DNA is added to the detector 10 and hybridized with the oligonucleotides at 35℃ for 4 hours or overnight.

3.5 The detector 10 is washed in 2x SSC/1% SDS solution at 35℃ for 15 minutes.

3.6 The detector 10 is washed in 0.2x SSC/0.1% SDS solution at 35 ℃ for 15 minutes.

3.7 The detector 10 is treated in 0.5% isolation reagent for 1 hour.

3.8 The detector 10 is treated with avidin-alkalinephosphatase for about 1 hour.

3.9 The detector 10 is washed in 1x PBST solution.

3.10 The detector 10 is washed in Tris/NaCl solution.

3.11 The detector 10 is treated with NBT/BCIP at room temperature to show the reacting dot in blue.

3.12 The blue dot having the specific oligonucleotide sequence presents the specific subtype of human papilloma viruses contained in the sample.

4. The detector 20 provided by the present invention is used for

identifying the subtypes of human papilloma viruses according to the following steps.

4.1 The PCR product having Cy5 labeled thereon is purified by PCR Clean Up-M System (Viogene, USA), and the PCR product is precipitated in ethanol. Then, the PCR product is dried.

4.2 The precipitated DNA is dissolved in 12 μl of the buffer (2x SSC/0.1% SDS), and centrifugated for 1 minute, and then placed on boiled water for 2 minutes. Then, the mixture is placed on ice for 5 minutes.

4.3 The mixture is centrifugated for 30 seconds, and 10 μl of the mixture is added to the left side of the dot array 22. A cover slice is carefully covered on the dot array from the left side of the dot array to prevent the bubble formation. Then, the detector 20 is place in Humid Chamber (Sigma, USA), and the dot array is faces downward at 35℃ for 4 hours or overnight.

4.4 The detector 20 is vertically placed in the solution A (2x SSC/1% SDS), and the detector is slightly oscillated apart from the cover slice. Then, the detector 20 is washed in a shaker at 160 rpm for 12 minutes.

4.5 The detector 20 is washed in the solution B (0.2x SSC/0.1% SDS) and oscillated at 35℃ for 12 minutes. The detector 20 is washed in water. Then the detector 20 is dried.

4.6 The dried detector 20 is scanned by GenePix^{TM}4000 (Axon, USA), excited by the light having 635 nm of wavelength, and analyzed by GenePixPro 3.0 (Axon, USA).

Please refer to Figs. 3(a) and (b). Fig. 3(a) is a schematic view showing the third embodiment of the present invention. The detector 30 provided by the present invention is used for simultaneously detecting

and identifying subtypes of human papilloma viruses contained in a sample. The detector 30 includes a carrier 31 and a dot array 32.

The carrier 31 is a nylon membrane. The actual length of the nylon membrane is about 1.44 cm and the actual width of the nylon membrane is about 0.96 cm. The dot array is mounted on the carrier 31 according to the foresaid method, wherein the distance between each dot is about 1.2 mm and the diameter of each dot is about 0.4 mm. Each dot is an oligonucleotide (15~30mer), and each oligonucleotide is used for specifically identifying a subtype of human papilloma viruses. The sequence of the oligonucleotide is selected from the Tables 1 to 36.

The subtype of human papilloma viruses identified by each dot of the dot array 32 is illustrated in Fig. 3(b). SC (system control) presents the PCR product amplified from any subtype of human papilloma viruses and biotin-contained primer. NC (negative control) presents the plants DNA fragment irrelevant to HPV. IN (internal control) presents the sequence 5'-gcccagactgtgggtggcag-3' of the housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAP-DH).

The plural detector 30 are used for identifying positive clones of human papilloma viruses according to the foresaid method, and the results are shown in Figs. 4(a) to (c). The positive clones are respective amplified by using MY11/MY09 primers and the first round of polymerase chain reaction. The products of the first polymerase chain reaction are analyzed in 2.5% agarose/EtBr, and the electrophoresis results are shown in Fig. 4(a). The products of the first round polymerase chain reaction are respective amplified by using GP5/GP6 primers and the second round of polymerase chain reaction. The products of the second polymerase chain reaction are analyzed in 2.5%

agarose/EtBr, and the electrophoresis results are shown in Fig. 4(b). The numbers labeled in Figs. 4(a) and (b) and the corresponding HPV clones are illustrated in Table 39.

Table 39

No.	HPV clone	No.	HPV clone	No.	HPV clone
M	DNA marker	7	HPV 33	14	HPV 56
1	HPV 6	8	HPV 35	15	HPV 59
2	HPV 11	9	HPV 44	16	HPV 61
3	HPV 16	10	HPV 45	17	HPV 66
4	HPV 18	11	HPV 52	18	HPV 70
5	HPV 26	12	HPV 53	19	HPV CP8061
6	HPV 31	13	HPV 54	20	HPV L1AE5

The foresaid products numbered 1 to 20 of the second polymerase chain reactions are respectively detected by the detectors 30, and the results are shown in Fig. 4(c). According to the comparison between the results shown in Fig. 4 (c) and Fig. 3(b) based on the "SC" dot, the detector 30 provided by the present invention is used for precisely identifying the subtype of human papilloma viruses. Furthermore, no cross reactions occur in the detection.

In addition, the detectors 30 are used for detecting and identifying the subtypes of human papilloma viruses contained in a sample, and the results are shown in Fig. 5. According to the comparison between the results shown in Fig. 5 and Fig. 3(b) based on the "SC" dot, HPV 53 is contained in the sample (1), HPV 45 is contained in the sample (2), HPV 52 is contained in the sample (3), and HPV 39 is contained in the sample (4).

Please refer to Figs. 6(a) and (b). Fig. 6(a) is a schematic view

showing the fourth embodiment of the present invention. The detector 40 is used for simultaneously detecting and identifying the subtypes of human papilloma viruses contained in a sample. The detector 40 includes a carrier 41 and a dot array 42. The carrier 41 is a glass plate, and the dot array 42 is mounted on the glass plate according to the foresaid method. Each dot is an oligonucleotide (15~30mer) for specifically identifying a subtype of human papilloma viruses. The sequences of the oligonucleotides are selected from Tables. 1 to 36. The subtype of human papilloma viruses identified by each dot of the dot array 42 is illustrated in Fig. 6(b).

The detector 40 is stained with SYBR Green II, scanned by GenePix™ 4000 (Axon, USA) and excited by the light having 635 nm of wavelength. The result is shown in Fig. 7(a).

In addition, the positive clones of HPV 11 and HPV 18 are respectively treated with two rounds of polymerase chain reactions, and the products of the polymerase chain reactions are respectively analyzed by the detectors 40. The results are shown in Fig. 7(b), wherein the red fluorescent dot presents positive result. According to the comparison between the results shown in Fig. 7(b) and Fig. 6(b), the detector 40 provided by the present invention is used for precisely identifying the subtype of human papilloma viruses. Furthermore, no cross reactions occur in the detection.

While the invention has been described in terms of what are presently considered to be the most practical and preferred embodiments, it is to be understood that the invention need not be limited to the disclosed embodiment. On the contrary, it is intended to cover various modifications and similar arrangements included within the spirit and

scope of the appended claims which are to be accorded with the broadest interpretation so as to encompass all such modifications and similar structures. Therefore, the above description and illustration should not be taken as limiting the scope of the present invention which is defined by the appended claims.